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Review

Skin Exposure Contributes to Chemical-Induced Asthma: What is the Evidence? A Systematic Review of Animal Models

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ABSTRACT

It is generally assumed that allergic asthma originates primarily through sensitization via the respiratory mucosa, but emerging clinical observations and experimental studies indicate that skin exposure to low molecular weight (LMW) agents, *i.e.* "chemicals," may lead to systemic sensitization and subsequently develop asthma when the chemical is inhaled. This review aims to evaluate the accumulating experimental evidence that adverse respiratory responses can be elicited upon inhalation of an LMW chemical sensitizer after previous sensitization by dermal exposure. We systematically searched the PubMed and Embase databases up to April 15, 2017, and conducted forward and backward reference tracking. Animal studies involving both skin and airway exposure to LMW agents were included. We extracted 6 indicators of "selective airway hyper-responsiveness" (SAHR)—*i.e.* respiratory responses that only occurred in previously sensitized animals—and synthesized the evidence level for each indicator into strong, moderate or limited strength. The summarized evidence weight for each chemical agent was graded into high, middle, low or "not possible to assess." We identified 144 relevant animal studies. These studies involved 29 LMW agents, with 107 (74%) studies investigating the occurrence of SAHR. Indicators of SAHR included physiological, cytological/histological and immunological responses in bronchoalveolar lavage, lung tissue and airway-draining lymph nodes. Evidence for skin exposure-induced SAHR was present for 22 agents; for 7 agents the evidence for SAHR was inconclusive, but could not be excluded. The ability of a chemical to cause sensitization via skin exposure should be regarded as constituting a risk of adverse respiratory reactions.

Keywords: Asthma, occupational asthma; chemicals; causes; skin; airway hypersensitivity; animal model

INTRODUCTION

Asthma is one of the most important lung diseases worldwide. To date, more than 400 causative agents, or asthmagens, have been identified to induce asthma in the workplace.[1]

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Occupational asthma can be induced through either immunological or irritant mechanisms. Some irritants may cause asthma that does not depend on a specific immunological reaction to the offending chemical, but such "irritant-induced asthma" will not be considered in this review.

A critical issue in asthma development relates to where in the body the immune response is initiated, *i.e.* where sensitization occurs. It is often taken for granted—as reflected by the terms "respiratory sensitizers" and "respiratory sensitization"—that the respiratory tract is the key route for sensitization. However, the assumption that sensitization necessarily occurs via inhalation can be challenged, not only because of the experimental evidence showing that respiratory responses can be elicited by exposing dermally sensitized animals via the airways, but also because of clinical and epidemiological studies indicating that the skin may be the site of initial sensitization.

"Asthmagens" can be classified according to their molecular weight (above or below 5,000 Da). Agents with high molecular weight (HMW) are (glyco)proteins of vegetal, animal or microbiological origin that can cause immunoglobulin E (IgE)-mediated allergy with mainly eosinophilic airway inflammation.[2] Agents with low molecular weight (LMW) are synthetic or natural chemicals and some metals that may act as haptens, binding to endogenous proteins to initiate an immunological response.[3] The mechanisms of asthma induced by sensitization to LMW agents have not yet been completely elucidated.[4] In such chemical-induced asthma, airway inflammation is usually characterized by a mixed neutrophil and eosinophil infiltration, and a specific IgE is not always essential.[5,6]

The first experimental study investigating the association between skin sensitization and airway hyper-responsiveness was conducted in the guinea pig with toluene diisocyanate (TDI) as the test agent.[7] Later, various other animal models of asthma demonstrated that skin exposure to various LMW chemical sensitizers, such as isocyanates and acid anhydrides, could lead to airway hyper-responsiveness upon elicitation via the respiratory route.[8,9] In these experimental models, "selectivity" of the airway response is crucial in that it implies that airway responses following respiratory administration of (a small amount of) a chemical can be elicited only in animals that have been previously sensitized via the skin to that chemical and not in control animals that have not been previously sensitized. Meanwhile, animal asthma models have been described in guinea pigs, rats and mice.

The paradigm that skin exposure to an LMW sensitizer can be at the origin of selective airway hyper-responsiveness (SAHR) is recognized to some extent, but not yet accepted widely. Moreover, this paradigm has not been adopted for regulatory purposes. When classifying chemicals, a strict distinction is made between skin (or dermal) sensitizers and respiratory sensitizers, with skin sensitizers defined as agents that may lead to contact dermatitis, and respiratory sensitizers defined as agents that may lead to airway hypersensitivity (mainly asthma) when inhaled.[10,11] In other words, for regulatory purposes dermal and respiratory sensitizers are defined according to the organ that is potentially affected by disease, but not to the site of initial sensitization. Several methods have even been developed to differentiate the skin sensitizers defined from respiratory sensitizers,[12] although it was accepted that several agents, such as isocyanates and acid anhydrides, can be both dermal and respiratory sensitizers.[13,15]

In this systematic review, we identified animal studies that applied skin exposure to LMW agents followed by airway challenge, and we compared 6 indicators of "SAHR" between groups

Table 1. The definition of terms associated with sensitization in this study

Term	Definition
Skin sensitizer	A substance that will lead to an allergic response following skin contact[11]
Respiratory sensitizer/asthmagen	A substance that will lead to hypersensitivity of the airways following inhalation of the substance[11]
Sensitizer	A substance that will lead to an allergic response, following the first phase of induction, and the second phase of elicitation, regardless of the site of exposure and reaction
Sensitization	An allergic process through the first phase of induction, and the second phase of elicitation, while the exposure sites in 2 phases are not necessary the same organ
Selective airway hyper-responsiveness	An airway response that is primed by either local or systemic exposure to a substance, and triggered by airway exposure to the similar substance

with and without prior skin exposure. The goal of our review was to reevaluate the validity of the traditional differentiation between respiratory and dermal sensitizers. One of the premises (and conclusions) of our review is that even when immune sensitization initially takes place in a specific organ (*e.g.* the skin), it has potential consequences for organs at a distant site (*e.g.* the airways). Consequently, the use of the traditional concepts of "skin sensitizer" and "respiratory sensitizer" may no longer be tenable. We have therefore used these terms only as used in the retrieved documents, and we have introduced the term "SAHR" to indicate an airway response primed by a previous (local or systemic) exposure to a chemical sensitizer, and triggered by airway exposure to the same (or a chemically similar) agent. The definition of terms associated with sensitization in this study is presented in **Table 1**.

METHODOLOGY

The methodology in this review was based on the Systematic Review Protocol for Animal Intervention Studies.[16] This study was not registered on PROSPERO database, since the literature suggests excluding animal studies on PROSPERO because the latter involve different methodologies and objectives.[17]

Study identification

We conducted a systematic literature search in MEDLINE/PubMed and Embase databases up to April 15, 2017 including non-English studies and all study types. Three groups of keywords were used: 1) the names of 347 LMW skin sensitizers and 192 LMW respiratory sensitizers (asthmagens), 2) respiratory symptoms/signs with related terms AND 3) dermatitis with related terms (**Supplementary Table S1**). We did not set limits on language or publication year.

The skin sensitizers were accessed from the local lymph node assay (LLNA) database,[18] because the LLNA is one of the gold standards to identify skin sensitizers.[12,19] The LMW respiratory sensitizers were accessed from an existing list of asthmagenic agents.[1] The CAS number of each agent was used to search for corresponding names in the PubChem database,[20] and these corresponding names were also included. These searching terms are detailed in **Supplementary Tables S2** and **S3**.

Study screening

The articles retrieved were combined and screened by 2 independent reviewers. The first reviewer (H.C.T.) reviewed all titles and abstracts by using the following inclusion criteria: 1) exposure to LMW agents; 2) either exposure through the skin or symptoms/signs of dermatitis; 3) either exposure through the airway or symptoms/signs of airway hyper-reactivity.

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After the first reviewer completed the screening, the articles included and an equal number of randomly chosen non-included articles were forwarded to the second one (S.R.). Cohen's kappa was calculated to assess the inter-rater reliability. In the case of discrepancy, consensus was reached by discussion and consultation of a third reviewer (B.N.).

Additional information source

After the relevant publications had been identified, articles involving animal studies were selected. The references and the citations of the articles were tracked by backward and forward snowball searching via the Scopus database. Duplicates, items that could not be retrieved, or items already included in the previous step were removed. The remaining items were then screened by the first and the second reviewer again, similar to the process described in study screening.

Data extraction and quality assessment

After relevant publications had been selected, the characteristics of each study were extracted, including animal species, animal sex, study designs, studied agents, exposure routes through skin and airway, reported outcome measures, and results.

To assess the quality of the animal studies selected, the Toxicological data Reliability Assessment Tool (ToxRTool) *in vivo* list was used,[21] which provided more detail than SYRCLE's risk of bias tool.[22] Based on the score from 21 criteria (**Supplementary Table S4**), studies were assigned to 1 of the 4 Klimisch score levels[23]: 1 (reliable without restrictions), 2 (reliable with restrictions), 3 (not reliable) and 4 (not assignable).

Assessment of the evidence level from outcome measures

The outcome measures extracted consist of alterations in skin draining lymph nodes, serum and 6 indicators of SAHR, which include 1) lung function measurements; 2) cytology in bronchoalveolar lavage (BAL); 3) inflammatory biomarkers (proteins, antibody or cytokines) in BAL; 4) pathology change in the airway or lung; 5) cytology in airway draining lymph nodes; and 6) inflammatory biomarkers (cytokines) in airway draining lymph nodes.

To determine whether a chemical leads to skin-induced SAHR, the results from 6 indicators of SAHR in each study were compared between the test group (receiving skin exposure and airway elicitation to the same test agent) and the control group (receiving skin exposure with control vehicle, and airway elicitation with test agent). Skin-induced SAHR was considered present if a significant difference ($P < 0.05$) between test and control groups was reported in the figure, table or text from the study.

The strength of evidence for each indicator was then synthesized from different studies via the modified Royal College of General Practitioners (RCGP) 3-star system (**Table 2**).[24,25]

Table 2. The modified Royal College of General Practitioners 3-star system for evidence assessment

Evidence level	Definition
★★★	Strong evidence: provided by generally consistent findings in multiple, high quality, scientific studies, based on at least 2 independent studies.
★★	Moderate evidence: provided by generally consistent findings in fewer, smaller or lower quality, scientific studies, based on at least 2 studies.
★	Limited evidence: provided by one scientific study or inconsistent findings in multiple scientific studies.
-	No scientific evidence: no conclusions can be drawn when there are not any studies that meet the criteria.

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Strong evidence (★★★) indicated skin-induced SAHR results from at least 2 independent study teams; moderate evidence (★★) indicated skin-induced SAHR results from at least 2 studies, which are not necessarily independent; and limited evidence (★) indicated skin-induced SAHR results from 1 study. An independent study team was defined as one without any overlap of contributing authors.

Finally, the summarized evidence weight of skin-induced SAHR for each chemical was categorized into 5 levels based on the approach of the Scientific Committee on Health, Environmental and Emerging Risks[26]: 1) high weight of evidence was given for a strong evidence (★★★) from 1 indicator of SAHR and 1 or more other lines of evidence without conflict; 2) middle weight of evidence was given for a moderate evidence (★★) from at least 1 indicator of SAHR; 3) low weight of evidence was given for limited evidence (★) from the primary indicators of SAHR; 4) weighing of evidence not possible to assess was concluded when no suitable evidence was available; and 5) uncertain weight of evidence indicated conflicting information from different lines of evidence.

RESULTS

The studies included and their characteristics

As noted in **Figure**, the initial literature search yielded 6,948 articles, of which 4,771 related to skin sensitizers and 3,525 related to LMW respiratory sensitizers (asthmagens). The first reviewer screened 6,948 articles based on the title and the abstract, identifying 457 potentially relevant articles. The articles identified by the first reviewer and another 457 randomly selected articles from the non-included articles were checked by the second reviewer without knowing the conclusion of the first reviewer. After the evaluation and comparisons by the second reviewer, 121 articles were removed from the 457 articles identified by the first reviewer, and 16 articles were added, yielding 352 articles, *i.e.* a Cohen's kappa coefficient of 0.62, with 81% agreement (**Supplementary Table S5**).

Among the 352 articles identified, 70 involving animal studies were selected to further track potentially relevant reports. This snowballing process yielded 2,308 articles, of which 448 were first removed, including duplicates, non-retrievable articles and already included articles. Then the first reviewer screened the 1,860 remaining articles and identified 72 potentially relevant articles. The second reviewer screened those 72 articles and an equal number of non-included tracked articles as previously described. A total of 74 additionally tracked articles were finally identified at this tracking step. The Cohen's kappa coefficient at this step was 0.93, with 97% agreement.

Characteristics of the animal studies selected

In total, 144 articles involving animal studies were included (**Supplementary Table S6**). These articles investigated the association between dermal exposure and respiratory effect, or between respiratory exposure and dermal effect. They were published in 49 different journals, the majority of which were toxicology journals (**Supplementary Table S7**).

The characteristics of the animal models in the selected studies are shown in **Table 3**. To evaluate the evolution over time, we employed an early period (before 2005), the recent period (from 2005) and the year 2005 to have approximately similar numbers of articles in each period. The most commonly studied animal type was mouse (n = 78 [54%]), followed

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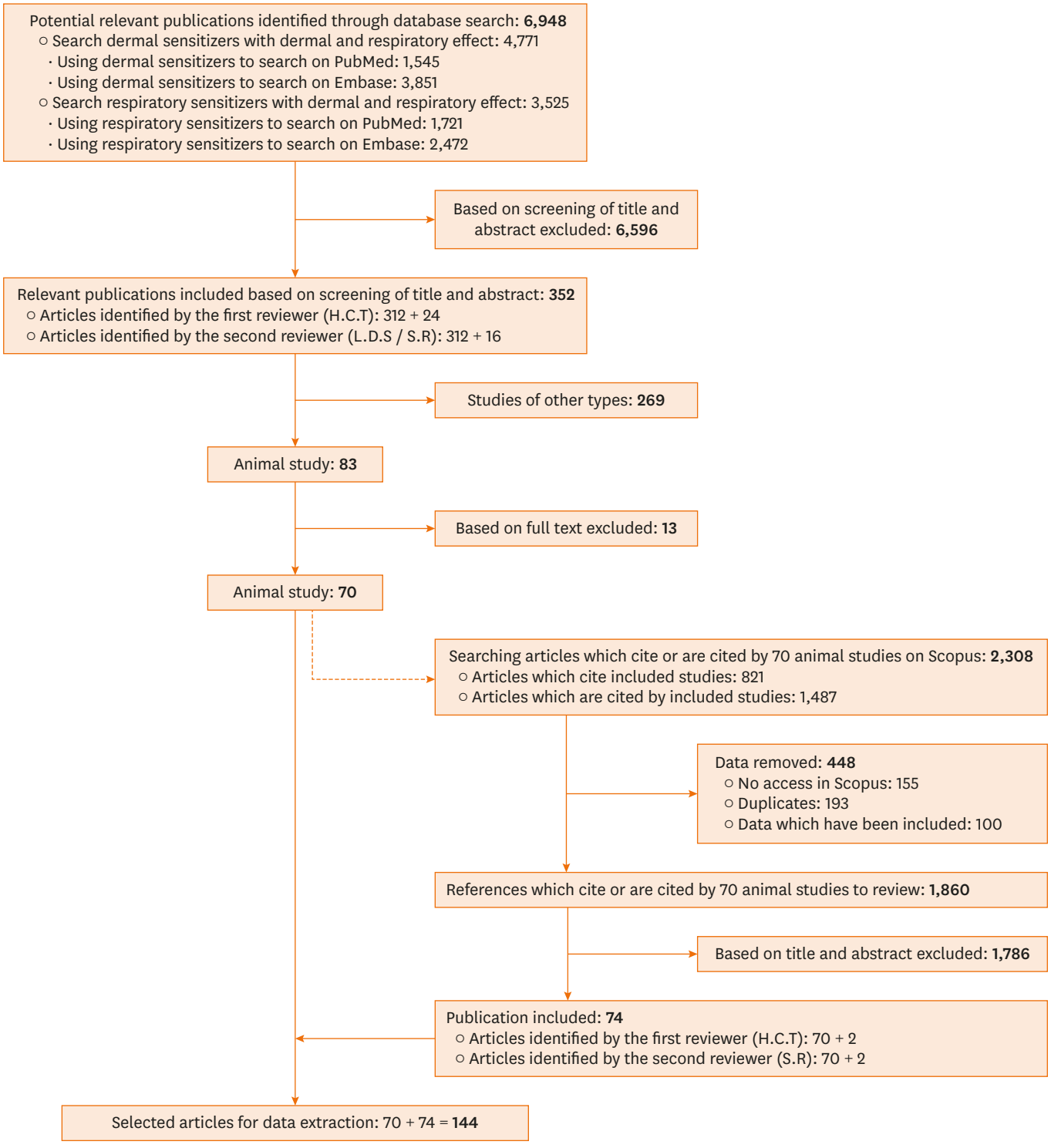

Figure. Flow diagram of systematic literature search.

by guinea pig and rat. In the recent period, no studies using guinea pigs or mice accounted for the majority (n = 48 [77%]). Male animals were used slightly more frequently than female animals.

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Table 3. Characteristics of animals and study designs

Characteristics	All studies (n = 144)	1981–2005 (n = 82)	2006–2017 (n = 62)
Animal species			
Mouse	78 (54)	30 (37)	48 (77)
Guinea pig	40 (28)	40 (49)	0 (0)
Rat	25 (17)	11 (13)	14 (23)
Hamster	1 (1)	1 (1)	0 (0)
Sex			
Male	76 (53)	42 (51)	34 (55)
Female	61 (42)	33 (40)	28 (45)
Both	4 (3)	4 (5)	0 (0)
Unreported	2 (1)	2 (2)	0 (0)
Design			
Skin exposure followed by airway elicitation	139 (97)	77 (94)	62 (100)
Airway exposure followed by skin elicitation	6 (4)	5 (6)	1 (2)

Values are presented as number (%).

Among the 144 articles, only 6 used airway exposure followed by skin elicitation, and the majority used the design of skin exposure followed by some form of airway elicitation (n = 139 [97%]). Detailed representative outcome measures are presented in **Supplementary Table S8**.

The quality assessment classified most of these studies (n = 98) in Klimisch level 1 (reliable without restrictions). All of the quality criteria, except for housing or feeding conditions, were fulfilled in more than 90% studies (**Supplementary Fig. S1**). The grading score of ToxRTool criteria for each study is provided in **Supplementary Table S9**.

Exposure routes

The specific routes and procedures of skin exposure and subsequent airway elicitation are presented in **Table 4**. The majority of studies in the early period (before 2005) used intradermal injection to induce skin sensitization (n = 31 [40%]), followed by epicutaneous applications simultaneously on the trunk and paws. However, these 2 methods have rarely

Table 4. Exposure routes of the studies identified

Method	All studies (n = 139)	1981–2005 (n = 77)	2006–2017 (n = 62)
Skin exposure			
Simple route			
Epicutaneous application on ear	41 (29)	3 (4)	38 (61)
Epicutaneous application on trunk	19 (14)	11 (14)	8 (13)
Epicutaneous application on trunk & paws	18 (13)	18 (23)	0 (0)
Epicutaneous occlusion on trunk	3 (2)	2 (3)	1 (2)
Intradermal injection	33 (24)	31 (40)	2 (3)
Toepad injection	2 (1)	2 (3)	0 (0)
Combination route			
Epicutaneous application on trunk, and later on ear	21 (15)	8 (10)	13 (21)
Cumulative contact enhancement test	3 (2)	3 (4)	0 (0)
Maximization test	1 (1)	1 (1)	0 (0)
Intradermal injection followed by epicutaneous application on ear	1 (1)	0 (0)	1 (2)
Intradermal injection followed by inhalation	2 (1)	1 (1)	1 (2)
Intradermal injection followed by intratracheal instillation	1 (1)	1 (1)	0 (0)
Airway elicitation			
Inhalation	57 (41)	34 (44)	23 (37)
Intranasal instillation	43 (31)	23 (30)	20 (32)
Intratracheal instillation	25 (18)	15 (19)	10 (16)
Intratracheal aerosol application	5 (4)	5 (6)	0 (0)
Pharyngeal aspiration	12 (9)	0 (0)	12 (19)

Values are presented as number (%).
Data are studies with the design of skin exposure followed and airway elicitation.

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been used in the recent period (from 2005). Approximately 75% of studies in the recent period used simple epicutaneous application for sensitization, either on the ear (n = 38) or on the trunk (n = 8); another smaller numbers of studies in the recent period exposed animals to chemicals via the trunk on the first day and then via the ear several days later.

In terms of airway elicitation methods, inhalation was most frequently used (n = 57 [41%]), followed by intranasal instillation, with little differences between the early and recent periods. Pharyngeal aspiration has been introduced as a new method in the recent phase (n = 12 [19%]), exceeding intratracheal instillation or intratracheal aerosol application during the same period.

Evidence weight for skin-induced SAHR

A total of 107 articles involving 29 chemicals investigated whether skin-induced SAHR exists. For all 29 chemicals, except 3-carene, EC3 values (concentration required to induce a 3-fold increase in lymph node cell proliferation in the LLNA) were available from the relevant database[18] or other references (**Supplementary Table S10**). The majority of the 29 chemicals proved to be extreme (n = 12) or strong (n = 8) skin sensitizers as defined according to the LLNA classification.[27]

Of the 6 articles with the design of airway exposure followed by skin elicitation, only 2—TDI[28] and dicyclohexylmethane-4,4′-diisocyanate (HMDI)[29]—showed that airway exposure led to selective skin response upon elicitation. The results of another 4 articles were inconclusive due to the lack of proper control groups.[30-33]

Table 5 shows the weight of evidence for 29 retrieved chemicals. Ten of them proved to be known human asthmagens, with definite experimental evidence of skin-induced SAHR for 8 of them, and insufficient evidence for 2 (cobalt chloride [$CoCl_2$] and 3-amino-5-mercapto-1,2,4-triazole [AMT]). Nineteen of the chemicals reviewed are unknown human asthmagens, but 14 of them do present evidence of skin-induced SAHR in experimental animals.

Table 5. Evidence weight of skin-induced selective airway hyper-responsiveness for reviewed chemicals

Skin sensitizer	Known asthmagen			Not known asthmagen		
	Chemicals	No. of studies	Evidence weight	Chemicals	No. of studies	Evidence weight
Extreme	Toluene diisocyanate	28	High	2,4-dinitrofluorobenzene	10	High
	Diphenylmethane diisocyanate	13	High	Dinitrochlorobenzene	10	High
	Hexamethylene diisocynate	4	High	Dicyclohexylmethane-4,4′-diisocyanate	2	Low
	Isophorone diisocyanate	1	Low	Oxazolone	2	Low
				2,4-dichlorophenoxyacetic acid	1	Low
				Dicarbonyl 4-oxopentanal	1	Low
				Meta-tetramethylene xylene diisocyanate	1	Not possible
				Methylisothiazolinone	1	Not possible
Strong	Trimellitic anhydride	35	High	Picryl (trinitrophenyl) chloride	6	Middle
	Hexahydrophthalic anhydride	2	Low	Ammonium hexachloroplatinate	1	Low
	Cobalt chloride	1	Not possible	BRP	1	Low
				P-tolyl(mono)isocyanate	1	Low
				Phthalic anhydride	1	Not possible
Moderate	4,4-methyltetrahydrophthalic anhydride	1	Low	Ammonium persulfate	3	Middle
	3-amino-5-mercapto-1,2,4-triazole	1	Not possible	Furathiocarb	1	Low
Weak	Furfuryl alcohol	1	Low	Piperidinyl chlorotriazine derivative	1	Low
				Methyl salicylate	1	Not possible
				Toluene-2,4-diamine	1	Not possible
Undetermined				3-carene	3	Middle

*Asthmagens are accessed from the list of Commission des normes, de l'équité, de la santé et de la sécurité du travail (CNESST).[1]

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Of all included chemicals, trimellitic anhydride (TMA) has been studied most often (n = 35), followed by TDI and diphenylmethane diisocyanate (MDI). Six chemicals were identified with high weight of evidence: 5 extreme skin sensitizers (2,4-dinitrofluorobenzene [DNFB], dinitrochlorobenzene [DNCB], hexamethylene diisocyanate, MDI and TDI); and 1 strong skin sensitizer (TMA). Three chemicals were identified with middle weight of evidence: picryl chloride, ammonium persulfate and 3-carene. Thirteen chemicals were identified with low weight of evidence. The evidence weight for each chemical did not change after removal of 9 unreliable studies with Klimisch score 3. It was not possible to weigh the evidence for 7 chemicals, including 2 extreme skin sensitizers (meta-tetramethylene xylene diisocyanate and methylisothiazolinone), 2 strong skin sensitizers ($CoCl_2$ and phthalic anhydride), 1 moderate skin sensitizer (AMT), and 2 weak skin sensitizers (methyl salicylate and toluene-2,4-diamine). Only 1 article was available for each chemical.

Detailed evidence strength for each indicator of skin-induced SAHR

Table 6 shows the evidence strength by RCGP system for 6 representative SAHR indicators. The detailed evidence summaries for individual SAHR indicators are provided in **Supplementary Tables S11-S16**. The majority of chemicals (n = 17) exhibit SAHR evidence

Table 6. Detailed evidence strength of skin-induced selective airway hyper-responsiveness for different chemicals

Chemical	No. of studies	Weight of evidence	Lung function	Cells in BAL	Cytokine, antibody or enzyme in BAL	Pathology of airway or lung	Cells in airway draining LN	Cytokines in airway draining LN
Extreme skin sensitizers								
Toluene diisocyanate	28	High	★★★	★★★	★★★	★	★★★	★
Diphenylmethane diisocyanate	13	High	★★★	★★★	★★★	★★	★★	★
2,4-dinitrofluorobenzene	10	High	★★	★★★	★★	★★	ND	ND
Dinitrochlorobenzene	10	High	★	★★★	★	-	-	-
Hexamethylene diisocynate	4	High	-	★★★	-	-	ND	ND
Dicyclohexylmethane-4,4′-diisocyanate	2	Low	★	ND	ND	ND	ND	ND
Oxazolone	2	Low	-	★	★	★	ND	ND
2,4-dichlorophenoxyacetic acid	1	Low	ND	★	★	ND	★	★
Dicarbonyl 4-oxopentanal	1	Low	★	★	ND	ND	-	★
Isophorone diisocyanate	1	Low	★	ND	ND	ND	ND	ND
Meta-tetramethylene xylene diisocyanate	1	Not possible	-	ND	ND	ND	ND	ND
Methylisothiazolinone	1	Not possible	-	-	ND	ND	ND	ND
Strong skin sensitizers								
Trimellitic anhydride	35	High	★★★	★★★	★★★	★★★	★★★	★
Picryl (trinitrophenyl) chloride	6	Middle	★★	ND	★	★	ND	ND
Hexahydrophthalic anhydride	2	Low	★	ND	ND	★	ND	ND
BRP	1	Low	ND	-	★	ND	-	★
Ammonium hexachloroplatinate	1	Low	★	★	-	ND	★	ND
P-tolyl(mono) isocyanate	1	Low	★	ND	ND	ND	ND	ND
Cobalt chloride	1	Not possible	ND	-	ND	-	ND	ND
Phthalic anhydride	1	Not possible	-	ND	ND	ND	ND	ND
Moderate, weak or undetermined skin sensitizers								
3-carene	3	Middle	★★	ND	ND	ND	ND	ND
Ammonium persulfate	3	Middle	★★	★★	★	ND	-	-
4,4-methyltetrahydrophthalic anhydride	1	Low	★	ND	ND	ND	ND	ND
Furathiocarb	1	Low	ND	-	★	ND	-	-
Furfuryl alcohol	1	Low	★	★	ND	-	ND	ND
Piperidinyl chlorotriazine derivative	1	Low	★	ND	ND	ND	ND	ND
3-amino-5-mercapto-1,2,4-triazole	1	Not possible	-	ND	ND	ND	ND	ND
Methyl salicylate	1	Not possible	-	ND	ND	ND	ND	ND
Toluene-2,4-diamine	1	Not possible	-	-	ND	ND	ND	ND

Reference for each category of each chemical is provided in **Supplementary Tables S11-S16**.
BAL, bronchoalveolar lavage; LN, lymph node; ND, no available data in this indicator; ★★★/★★/★/-, strong/moderate/limited/no scientific evidence under Royal College of General Practitioners 3-star system.

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based on alterations in lung function parameters, including rising Penh, resistance, tissue damping, elasticity, and elastance. Specific changes in respiratory pattern were also observed and enhanced responses to methacholine challenge were also frequently observed (**Supplementary Table S11**).

In BAL fluid, evidence for SAHR was provided by rising ratios of neutrophils or eosinophils (**Supplementary Table S12**), other indicators of inflammation, such as changes in more than ten cytokines or enzymes (**Supplementary Table S13**), or increased total and specific IgE (for TMA and 2,4-dichlorophenoxyacetic acid).

In lung or airway tissues, pathological changes indicative of skin-induced SAHR varied from pulmonary edema as evidenced by plasma protein exudation, to infiltration by neutrophils or eosinophils, peribronchiolar or perivascular cell accumulation, epithelial damage, and laryngitis (**Supplementary Table S14**). In airway draining lymph nodes, cellular responses included increased B cells, increased dendritic cells, and influx of eosinophils (**Supplementary Table S15**) and/or changes in secretion of interleukin (IL)-4, IL-10 and IL-13 (**Supplementary Table S16**).

DISCUSSION

This systematic review provides a comprehensive and critical assessment of experimental studies investigating if LMW agents are capable of inducing SAHR after having been applied to the skin. To our knowledge, this is the first evidence-based analysis in this field. Our review found 29 LMW agents showing the ability to cause skin-induced SAHR with high evidence for 6 chemicals, middle evidence for 3 chemicals, and low evidence (due to few independent studies) for 13 chemicals. This finding supports the paradigm that the skin may serve as an important route of exposure for asthma development.[15]

The current review provides evidence that directly links skin exposure to subsequent airway hyper-responsiveness. Almost all of the chemicals reviewed, except 3-carene (also classified as a significant contact allergen),[34] had available positive LLNA results. The evidence is mostly seen for extreme or strong skin sensitizers, but not necessarily limited to these categories. Moderate or even weak skin sensitizers were also found to generate skin-induced SAHR.[35,36] The evidence weight for 7 chemicals proved not possible to assess because of limited study number. However, we cannot exclude the possibility that some of these agents could still lead to skin-induced SAHR under different doses or frequencies of sensitization or elicitation.

Ten of the agents included are known asthmagens for humans, and 8 of them present evidence of skin-induced SAHR. Of the other 19 agents which are not known as asthmagens, such as DNCB and DNFB, 14 have been demonstrated to be capable of developing skin-induced SAHR in animals (**Table 5**). The evidence is based mainly on lung function change and inflammatory responses in BAL. This phenomenon indicates that dermal and respiratory sensitizers are not mutually exclusive even though methods have been developed in an attempt to differentiate these 2 groups.[37,38]

It is noteworthy that the majority of studies included in this review have been published in toxicology journals (**Supplementary Table S7**), thus suggesting insufficient interest and recognition of this clinically and mechanistically relevant issue by respiratory medicine, dermatology and immunology.

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Implications

In humans, many common contact allergens have also been identified as asthmagens,[39,40] and occasionally some patients have both allergic contact dermatitis and asthma caused by the same agent.[41,42] Moreover, several epidemiological studies showed an association between skin and respiratory symptoms in the workplace.[43] For instance, an association between skin exposure to MDI and asthma-like symptoms has been demonstrated.[44] Interestingly, in a workforce exposed to di-isocyanates, the prevalence of chemical-induced asthma did not decrease despite reductions in aerial exposure via better collective and personal protective equipment.[14,45]

In this review, we found that chemicals not generating obvious clinical respiratory symptoms in epidemiological studies, such as DNCB, may still induce subclinical airway responses in *in vivo* studies. Thus, current methods for defining respiratory sensitizers, which are based mainly on human studies, might neglect potential hazards. According to the evidence that skin exposure may lead to a change in airway susceptibility, monitoring merely the ambient levels of respiratory sensitizers in the environment may underestimate the hazards spread through different exposure routes.

The relationship between skin sensitization and asthma also has applications in diagnostic practices. For example, skin prick testing (SPT) has been used for decades to demonstrate sensitization to an allergen by provoking an immediate IgE-mediated allergic response through introducing the suspected allergen into the skin's surface with a needle.[46] In the updated scheme for the diagnosis of asthma in the workplace, SPT is indicated as one of the first-line methods that should be applied.[47] However, SPT is used almost exclusively for HMW agents, and its validity is very low (though not entirely absent) for most LMW agents. On the other hand, the use of skin patch testing, which is the gold standard for demonstrating sensitization to LMW chemicals in patients with contact dermatitis, has not been included in the regular etiological diagnosis of chemical-induced asthma.[47]

Relevance

Throughout this review, we have examined the effects from LMW agents. Compared with LMW agents, animal studies about HMW agents in the skin-airway relationship have been less thoroughly evaluated. The agents studied in recent decades included ovalbumin,[48-53] house dust mite[54,55] and latex.[56,57] The methods used for inducing sensitization to HMW agents through skin exposure usually needed either a continuous exposure for more than 72 hours, a skin injury or an intraperitoneal injection. These exposure methods indicate that it is more difficult for HMW agents to enter the skin and initiate subsequent immune reactions. This may also be reflected in the LLNA database for skin sensitizers, which contains only 1 HMW agent (ovalbumin).

This review focused on animal models, which remain important methods to determine whether a chemical is able to elicit airway responses. Even though several techniques have been developed to replace *in vivo* asthma models,[58,59] these techniques are less suitable for investigating the effects of different exposure routes. Compared with *in vitro* studies, *in vivo* studies can mimic better the interplay between different chemicals and physiological conditions. Some findings in animals after LMW agent exposure may share similar mechanisms as human respiratory effects.[60]

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Challenges and limitations

During the process of this review, we faced several challenges. One challenge in connecting skin exposure and asthma development is that no widely acknowledged methods exist to identify respiratory sensitizers for animal models to date,[11,61] and the identification mainly relies on human data.[12] Therefore, we used 6 parameters in this review to identify the evidence of SAHR.

Another challenge is that several variables may alter the effects of chemicals. These variables include animal strain, routes of exposure, and frequencies and dosages of sensitization and elicitation.[62] There is no harmonized system to integrate these variables, and thus a formal meta-analysis from the selected studies was not feasible. In addition, it is difficult to simplify the dose-response curve of the allergic effect, because higher exposures in the induction phase may result in lower potency of sensitization.[63] The overdoses of airway elicitation may also result in a direct irritant effect, which elicit the process and effect of the sensitization.

In this review, the second reviewer only screened a selected number of studies (n = 914 [13%] in the first screening stage; n = 144 [7.8%] in the second screening stage). This method might increase the false negative rate through excluding some potentially relevant studies. However, we limited the false negative rate through forward and backward reference tracking. Other researchers have validated this method by comparing full systematic reviews with 20% enhanced rapid reviews and concluded that the latter method identified similar numbers of relevant studies.[64]

Mechanistic considerations

While the pathogenesis of skin and airway sensitization has been extensively studied and some focuses have been put on immunological cells in lung after skin exposure,[65] the process from skin exposure to asthma development has not yet been fully delineated.

Based on the existing scientific findings, we hypothesize the following process which links the skin and airway. The skin sensitization begins when haptens penetrate the striatum where the haptens become linked to proteins or macromolecules, sensed by Langerhans cells, and presented to T cells at skin draining lymph nodes.[66,67] Once the T cells are activated, they enter the circulatory system[61,68] and may then reach the airway tissue or its draining lymph node. Upon the re-exposure to haptens, dendritic cells in the airways can re-stimulate the resident T cell and initiate asthma development.[4,69,70] Accordingly, it would be valuable to design experiments that identify and track different cells covering the critical pathway after skin exposure.

CONCLUSION

This review systematically identified studies investigating the association between skin exposure to chemicals and airway hyper-responsiveness in experimental animal models. Although our systematic review of the experimental evidence does not answer the questions as to what makes a chemical an allergen and what are cellular events driving the allergic response and target organs, the available evidence suggests that most of the studied LMW chemicals have the potential to induce SAHR via previous skin exposure. Admittedly, some agents possess only partial evidence mainly because complete outcome measures for each agent have not yet been fully investigated. Studies about TDI, TMA and MDI attracted most attention, while more than

half of the LMW agents have been individually tested only once. Despite their small number, studies with the design of airway exposure followed by skin elicitation also support the concept that the immunological effects from the skin and airway interact with each other. Namely, airway exposure leads to selective skin response upon elicitation.

By doing this, we conclude that sensitization is not only an issue of specific organs but also an issue of the immune system in all organs, and the response is dependent on the route of elicitation. Therefore, chemicals that have been identified as skin sensitizers in a series of tests should still be carefully considered able to induce airway adverse response once inhaled because several skin sensitizers also share the characteristics of respiratory sensitizers, although we acknowledge that this concept may not be shared by all experts.[71,72] We believe the latter view is to a large extent due to the fact that many skin sensitizers are only rarely or never inhaled. Moreover, we do not dispute that intrinsic chemical properties other than volatility can render some sensitizers more likely to cause dermal disease rather than respiratory disease, and *vice versa*. However, these chemical determinants are still largely unknown. Accordingly, skin exposure should be taken into account by clinicians dealing with asthma or by surveillance programs in the environment. Furthermore, it cannot be excluded that every chemical sensitizer may elicit the respiratory response upon inhalation, regardless of the route of sensitization.

The exhaustive evidence in this review also brings the concept of "distant sensitization" into asthma development, which means the susceptibility of the airways to a chemical agent may result from prior exposure distant from the airways. This provides an opportunity to reconsider the hazard definition of skin or respiratory sensitizers. The traditional view defined respiratory sensitizers simply as agents that induce airway sensitization via airway exposure. Based on our review, however, this definition is not appropriate for classifying chemicals accurately, since the consequences of sensitization are not necessarily restricted to a specific organ. A chemical may induce respiratory disease after sensitization via dermal contact even when the ambient level is too low to cause sensitization via the respiratory mucosa.

Therefore, we propose to consider the ability of a chemical to cause immune sensitization as a generic property, regardless of the site of sensitization and elicitation of symptoms, by analogy with the ability to cause cancer. Obviously, in terms of "risk"—which depends on both hazard and exposure—and its management, the type of exposure must be taken into account. While in terms of "hazard," chemicals are considered (and labelled) as carcinogens, regardless of exposure routes or target organs.[11] Thus, an agent that causes immunological sensitization should also simply be labelled as "sensitizer."

In terms of risk assessment, the important issue is whether the offending agent can cause immunological sensitization and whether it can reach the airways. The initiation of the sensitization process and subsequent manifestations depend at least on 1) chemical properties and co-exposures; 2) the mode of exposure; and 3) individual tissue characteristics (*e.g.* accessibility of antigen-presenting cells across a damaged epithelium). Consequently, if a chemical can lead to allergic contact dermatitis, we should assume that the chemical may also cause asthma or other types of pulmonary hypersensitivity reactions when inhaled by a sensitized person.

To understand the mechanism of airway sensitization, further investigations are warranted with a focus on the pathological change in airway and associated immunological pathways.

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Lack of established criteria for sensitization in the airways increases the difficulty in analyzing the evidence, and future studies are required to understand the key events of asthma development in order to clarify the criteria. A harmonized system is warranted to construct the model of asthma development.

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SUPPLEMENTARY MATERIALS

Supplementary Fig. S1
Toxicological data Reliability Assessment Tool quality assessment summary.

Click here to view

Supplementary Table S1
Terms used to identify relevant studies with respiratory and skin effect

Click here to view

Supplementary Table S2
Skin sensitizers used to identify relevant studies

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Supplementary Table S3
Respiratory sensitizers used to identify relevant studies

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Supplementary Table S4
Reliability assessment criteria of toxicological studies (*in vivo*)

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Supplementary Table S5
Comparison of the screening between 2 reviewers

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Supplementary Table S6
List of 144 included studies for data extraction

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Supplementary Table S7
Top 15 journals with the most articles

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Supplementary Table S8
Reported outcome measures in studies adopting skin exposure followed by airway elicitation

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Supplementary Table S9
The grading score of Toxicological data Reliability Assessment Tool criteria for studies investigating skin-induced selective airway hyper-responsiveness

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Supplementary Table S10
Categorization of reviewed chemicals based on LLNA EC3 value

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Supplementary Table S11
Strength of evidence for changes of lung function

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Supplementary Table S12
Strength of evidence for changes of cells in bronchoalveolar lavage

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Supplementary Table S13
Strength of evidence for changes of protein, antibody or cytokine in bronchoalveolar lavage

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Supplementary Table S14
Strength of evidence for changes of pathology or histology quantification of airway

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Supplementary Table S15
Strength of evidence for changes of cells in airway draining lymph node

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Supplementary Table S16

Strength of evidence for changes of cytokines in airway draining lymph node

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Supplementary Data S1

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